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## Occurrence of Puma Lentivirus Infection in Cougars from Washington

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**ABSTRACT:** Puma lentivirus (PLV) antibodies were detected in 13 (25%) of 52 serum samples obtained from cougars (*Felis concolor*) collected by hunters. The serum samples were collected from November 1993 through January 1994 from four specific regions throughout the state of Washington (USA), and included the Olympic Mountains, the Cascade Mountains, the Blue Mountains, and the Selkirk Mountains. More (38%) seropositive cougar samples originated from the Cascade Mountains than from any other site. The overall seroprevalence for PLV infection in Washington cougars was higher than previously reported for cougars sampled in Oregon and Idaho (USA), but lower than in cougars sampled in Arizona, Colorado, and California (USA).

**Key words:** Cougar, *Felis concolor*, puma lentivirus, feline immunodeficiency virus, viral ecology.

We have been studying the potential impact of infectious microorganisms on cougar (*Felis concolor*) populations (Glass et al., 1994; Roelke et al., 1993). During the course of our serologic survey of cougars in the northwestern United States we detected antibodies to puma lentivirus (PLV) in populations of cougars sampled by hunters throughout the state of Washington. The occurrence of PLV infection in Washington cougars had not been previously reported, but has been recognized in the states of Oregon (USA) and Utah (USA), as well as in Canada (Olmsted et al., 1992).

The lentiviruses comprise retroviruses that have protracted periods of latency (no cellular expression of virus), which may last for years without demonstrable clinical signs in affected animals and humans (Fenner, 1993). Notable among this group of viruses are maedi-visna virus of sheep, equine infectious anemia virus, caprine ar-

thritis-encephalitis virus, bovine lentivirus, feline immunodeficiency virus (FIV), and human immunodeficiency virus (Narayan et al., 1988; Dua et al., 1994; Pedersen and Torten, 1995).

Pedersen et al. (1987) initially reported isolation of feline lentivirus from a colony of domestic cats experiencing multiple secondary viral and bacterial infections of a recurrent nature despite eradication of feline leukemia virus (FeLV) from the population. The virus was subsequently named feline immunodeficiency virus due in part to transient immune suppression after experimental inoculation. The blood cell profile reflects transient leukopenia with neutropenia appearing as early as 14 days post-inoculation and returning to normal values by 56 days post-inoculation (Dua et al., 1994). Attempts to induce life-threatening disease with FIV in specific pathogen free (SPF) cats have been for the most part unsuccessful. However, English et al. (1994) recently reported that FIV-naturally infected domestic cats had more severe opportunistic infections than SPF cats, suggesting that cofactors were involved in the progression of disease, and that the disease advanced through stages in cats similar to HIV in humans (Pedersen and Torten, 1995).

Following reports of FIV in domestic cats, there have been several studies reporting on the occurrence of FIV or closely related lentiviruses in captive and free-ranging nondomestic felids based on serologic studies. Barr et al. (1989) reported the serologic occurrence of FIV antibodies in two of 17 captive snow leopards (*Panthera uncia*), one of six captive lions (*Panthera leo*), and one of six captive tigers (*Panthera tigris*).

*era leo*), and six of 20 Florida panthers (*Felis concolor coryi*). Letcher and O'Conner (1991) surveyed a captive population of Asian lions (*Panthera leo persica*) and detected 16 of 22 seropositive to FIV using a commercially available enzyme linked immunosorbent assay (ELISA, Idexx Laboratories, Inc., Westbrook, Maine, USA). All the ELISA seropositive samples were subsequently verified by the more specific Western blot serologic assay (Letcher and O'Conner, 1991).

In 1992, a worldwide seroprevalence study was reported by Olmsted et al. (1992). Their results of a large survey on cougars supported the earlier observations of Barr et al. (1989) and of Letcher and O'Conner (1991). Olmsted et al. (1992) reported an overall seroprevalence to PLV in cougars of 35%, with a range between 0% in Idaho (USA) ( $n = 3$ ) and 80% in Arizona (USA) ( $n = 10$ ). Based on these results, and those of Langley et al. (1994), Carpenter and O'Brien (1995) speculated that PLV and FIV are rather antiquated felid viruses whose genomic divergence had proceeded primarily within separate species and subspecies, with interspecies exchange of virus being rare. In this report we present results from testing cougars sampled throughout Washington for PLV and another retrovirus, FeLV, and present some perspectives on the importance of PLV infection in wild felids.

Blood samples from free-ranging cougars were obtained from hunters working in conjunction with the Washington Department of Fish and Wildlife, Olympia, Washington. Serum was separated from the whole blood submitted and stored at  $-20\text{ C}$  until ready for testing. Estimated age based on dental pattern, sex and capture location of the cougars were recorded. Cougars were sampled from four specific mountain regions of Washington (Fig. 1): the Olympic Mountains ( $47^{\circ}25'$  to  $48^{\circ}5'N$ ,  $123^{\circ}0'$  to  $124^{\circ}30'W$ ), Cascade Mountains ( $46^{\circ}30'$  to  $49^{\circ}0'N$ ,  $119^{\circ}30'$  to  $122^{\circ}0'W$ ), Blue Mountains ( $46^{\circ}0'$  to  $46^{\circ}35'N$ ,  $116^{\circ}55'$  to  $118^{\circ}30'W$ ) and Selkirk

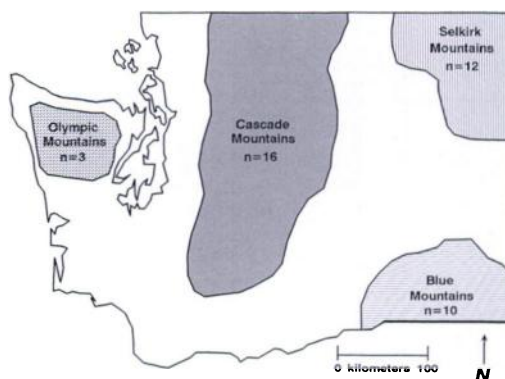


FIGURE 1. Map depicting the geographic areas within Washington state from which blood samples from cougars (*Felis concolor*) were collected.

Mountains ( $48^{\circ}0'$  to  $49^{\circ}0'N$ ,  $117^{\circ}03'$  to  $118^{\circ}30'W$ ). Although the four mountain ranges are geographically distinct from one another (Fig. 1), only the Olympic Mountains are restricted to Washington. The Cascade and Selkirk Mountain ranges extend north into Canada, and the Blue Mountain range extends south into Oregon.

Serum samples were tested for antibodies to PLV by a commercially available ELISA (Idexx Laboratories, Inc.) licensed for detection of antibodies to FIV. The serologic test methods were run as described by Roelke et al. (1993). The FIV ELISA has been reported to detect cougars infected with PLV with high specificity (Barr et al., 1989). Lacheretz et al. (1995) reported 100% correlation between the FIV ELISA and FIV Western blot. The serum samples were also tested for feline leukemia virus (FeLV) using a commercially available ELISA (Idexx Laboratories, Inc.), which was used to detect the p27 core antigen of FeLV.

Cougars had antibodies to PLV, with a range in the seroprevalence from 0% in the Olympic Mountains ( $n = 3$ ) to 38% ( $n = 16$ ) in the Cascade Mountains (Table 1). The Selkirk Mountains had 33% (four of 12), followed by the Blue Mountain area with 10% (one of 10). All the cougars tested negative for FeLV p27 antigen.

Although the sample size was limited

TABLE 1. Summary of testing for feline immunodeficiency virus/puma lentivirus and feline leukemia virus infections in Washington cougars.

Location	Number tested	FIV <sup>a</sup> (%)	FeLV <sup>b</sup>
Olympic Mountains	3	0/3 <sup>c</sup> (0%)	0/3 <sup>d</sup>
Cascade Mountains	16	6/16 (38%)	0/16
Selkirk Mountains	12	4/12 (33%)	0/12
Blue Mountains	10	1/10 (10%)	0/10
All samples identified to location	41	11/41 (27%)	0/41
All samples	52	13/52 (25%)	0/52

<sup>a</sup> FIV, feline immunodeficiency virus antibody detection by ELISA.

<sup>b</sup> FeLV, feline leukemia virus antigen detection by ELISA.

<sup>c</sup> Number seropositive/total number sampled.

<sup>d</sup> Number FeLV p27 Ag positive/total number sampled.

from some regions, we observed PLV infection patterns that were consistent with prior investigations in free-ranging cougars (Olmsted et al., 1992; Roelke et al., 1993; Paul-Murphy, 1994). The overall seroprevalence for PLV infection in cougars in Washington (25%) was higher than in Oregon (9%,  $n = 11$ ) and Idaho (0%,  $n = 3$ ), but lower than Arizona (80%,  $n = 10$ ), Colorado (USA) (67%,  $n = 9$ ), and California (USA) (56%,  $n = 16$ ) (Olmsted et al., 1992). Within Washington there was variation noted between the areas (Table 1). Similar variation was noted in cougar populations in California from two independent studies (Olmsted et al., 1992; Paul-Murphy et al., 1994) which had a range between 0% and 56%. These observations support contentions by Brown et al. (1993) that PLV is enzootic in certain populations of cougars and absent in others. Based upon the difficulty of transmitting FIV, other than by cat bite or other forms of horizontal blood-borne spread (Pedersen and Torten, 1995), we agree with Brown et al. (1993) that PLV infection occurred after the geographic separation of the selected cougar populations. This would further support the hypothesis that the spread of PLV into other seronegative populations may be restricted by natural geographic barriers (Brown et al.,

1993). It is unknown whether the prevalence of PLV in cougars will increase in those areas where urbanization and human encroachment into cougar habitats has occurred, because of the potential for increased contact between FIV-infected domestic cats and susceptible cougars.

Based on this study we have raised some questions about the ecology of PLV in wild cougar populations such as: are seropositive cougars infectious to other cougars and other felids, such as domestic cats; and what are the long term consequences of PLV infection upon cougar populations? Rigby (1995) reported on multiple strains of feline lentivirus in domestic cats with variable levels of virulence, ranging from avirulent to virulent, with severe alterations of T-cell populations. If this were indeed the case, then only a subset of feline lentiviruses, those strains adversely affecting the immune system, should be referred to as FIV, and the remainder of the strains, which are avirulent, should be referred to as feline lentivirus (FLV). However, classification of the viruses based upon their virulence may be imprecise due to the frequency of mutation for the lentiviruses (Langley et al., 1994). Although free-ranging felid populations with antibodies that react to FIV have been detected in a number of serologic surveys, the assumption that the viruses inducing these antibodies are the same or will result in an alteration of the immune response is conjecture (Spencer et al., 1992; Brown et al., 1993). Just the opposite observation from FIV infection in domestic cats has been made for both the lion and cougar. A high percentage of populations of lions and cougars have serologic evidence of exposure to a lion lentivirus and PLV, respectively, without any clinical signs (Olmsted et al., 1992; Callanan, 1995; Carpenter and O'Brien, 1995). The observation that lentivirus exposure in free-ranging felids is more common than in domestic cats to evidence that infection does not necessarily lead to, nor contribute to, fatalities (Brown et al., 1994; Langley et al., 1994).

If the lentivirus infections of lions and cougars has evolved within these particular species, then avirulent variants of the respective lentiviruses may well have been selected for as a form of natural immunization against more virulent strains of the respective virus (Carpenter and O'Brien, 1995). Further studies are in progress in order to determine the full extent of felid lentivirus infection in free-ranging cougars in the northwest, and the potential for interspecies transmission to other members of the feline family.

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